## Estimating injury to nearshore fauna resulting from the Deepwater Horizon Oil Spill

#### **Technical Report**

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### Edits to "Estimating injury to nearshore fauna resulting from the Deepwater Horizon Oil Spill"

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- (1) Minor changes were made to Figure 19 to reflect updated information from the toxicity investigators on (Juvenile amphipod (*Leptocheirus plumulosus*) mortality after a 10-day exposure to sediments spiked with Deepwater Horizon oil in the laboratory).
- (2) Changes were made to the total injury estimate of amphipods as a result of minor changes to the dose-response curve (Figure 19). The biomass loss estimate changed from 407 MT to 382 MT.
- (3) Updated Tables 22 and 24 as a result of minor changes to the dose-response curve (Figure
- 19). Numbers changed reflecting a range of survival reductions from 35-95% instead of 37-98%
- (4) Updated Figure 20 as a result of minor changes to the dose-response curve (Figure 19) (reflecting changes in survival in each category).
- (5) In the Red Drum section, the size of fish used in the toxicity experiment was updated to correct incorrect information in the draft report. The size of the fish was changed from 3.5 cm to 2.3-3.4 cm juvenile Red Drum.
- (6) Updated Table 15-18, and Figure 15 and 16 to remove injury from 2013 due to revised dose response/chemistry data received from the Toxicity investigators.
- (7) The text was changed to indicate that marsh soil conditions had dropped below toxic concentrations before 2013. The EC-20 value changed from 31 ppm tPAH to 37 ppm tPAH due to revised chemistry data collected by the Toxicity investigators.

(8) Text and Table 19 were changed to indicate that Red Drum production lost was changed
from 639 MT to 563 MT.

#### Summary

The vast quantity of contaminants (crude oil, dispersants, natural gas) released and introduced into one of the world's most productive ocean basins, the geographic scope of shoreline oiling (100's of km of sandy and vegetated shorelines), and the duration of exposures (> 100 days of oil in surface waters) represents an unprecedented challenge for marine ecologists to estimate the injury resulting from the 2010 Deepwater Horizon Oil Spill in the northern Gulf of Mexico. The enormous scale of the oil spill prevented any comprehensive field-based study of the ecological impacts, and the sampling designs for existing baseline data, which historically were collected in support of fisheries stock assessments, was never intended to achieve sufficient statistical power to be used in the context of environmental impact assessment. To estimate injury, we used the results of geographically focused field studies that used oiling categories along vegetated shorelines as oil exposure gradients or laboratory based toxicity studies using field collected or oiled spiked sediments to assess lethal or sublethal (growth, fecundity) endpoints for the seven taxa. Nevertheless, we chose for assessment seven abundant and wide-ranging taxa that are integral to nearshore, vegetated shoreline habitats in the northcentral Gulf of Mexico and represent a broad taxonomic and trophic range: Amphipods (Order: Amphipoda), Brown Shrimp (Farfantepenaeus aztecus) and White Shrimp (Liptopenaeus setiferus), Southern Flounder (Paralichthys lethostigma), Gulf Killifish (Fundulus grandis), Marsh Periwinkle (Littorina littoraria), and Red Drum (Sciaenops ocellatus). Substantial decreases in secondary production (50-90% decline) would be expected for amphipods, marsh periwinkles, brown and white shrimp, Red Drum and Southern Flounder in areas adjacent to vegetated shorelines that experienced Heavy Persistent oiling compared to shoreline areas that had no observed oil; shorelines with intermediate levels of oiling also experienced reduced secondary production for

Brown and White Shrimp as well as amphipods. In addition to loss of secondary production, the one species for which fecundity studies were performed (*Fundulus grandis*) indicated substantive (>50%) reduction in hatching success of eggs in Heavy Persistent Oiled areas.

Duration (2 to 4+ yrs) of the estimated losses of the ecosystem service of secondary production varied by taxon and our assessment capability was heavily influenced by limitations of the individual studies. Our results nonetheless indicate significant loss in this ecosystem service occurred from the oiling of 100's of kilometers of vegetated shoreline in the northcentral Gulf of Mexico and that substantive restoration activities will be required to compensate for the loss.

Keywords: *Natural Resource Damage Assessment*, saltmarsh, oyster reefs, Deepwater Horizon *Oil Spill, Injury, Ecosystem Services, Environmental Impact*.

#### **Background**

Shoreline ecosystems are often the repository of contaminants released into oceans. The high biological productivity of this shallow-water, nutrient-rich environments implies that contaminants deposited in these environments can have substantive negative effects on the ecological goods and services that humans expect from these systems. The negative effects of oil spills on nearshore habitats are well established and include lethal as well as a range of sublethal endpoints (see NRC 2003 for a review). While individual responses to the toxic components of oil can account for substantial changes in survivorship, growth, fecundity and other metrics of plant and animal health, oiling of foundational species or ecosystem engineers, many of which form biogenic habitat, may result in more pervasive and long-lasting direct and indirect negative effects (e.g., Peterson et al. 2003; Powers et al. in review). In addition to the effects of direct oiling (physical smothering and toxicity), shoreline response activities during and after the spill intended to prevent or mitigate the effects of oil also are known to induce negative impacts on habitat-forming species (Driskell et al. 2000, Martinez et al. 2012, Peterson et al. 2012). The combination of immense geographic (100's of km of shoreline oiled) and temporal scope (active oil release for over 100 days) of the Deepwater Horizon Oil Spill (DWHOS) is unprecedented in U.S. experience and implies potential for elevated impacts in the northern Gulf of Mexico spill region.

The wide spatial and long temporal scales of the DWHOS prevented a comprehensive ecosystem-based study of injury to natural resources. Even if one were to focus exclusively on nearshore ecosystems, ignoring the effects on offshore pelagic, mesopelagic, and deep-water benthos as well as on a diverse suite of estuarine habitats and species, a comprehensive ecosystem study would need to encompass an area from Texas to Florida (1,000s of km), sample

across years in time, and sample multiple trophic levels at appropriate spatial and temporal scales. The lack of any rigorously and/or systematically collected ecosystem baseline samples, which proved impossible because the scale of the spill was not known until well into the event, prevented a Before-After Control-Impact (BACI) design study (Stewart-Oaten et al. 1986, Underwood 1991). Further, available baseline data to inform a similar approach are either extremely geographically restricted (individual researcher studies) or collected as part of fisheries analyses, which were never designed to have the statistical power to detect the effects of episodic events or determine absolute density of animals. The absence of rigorous "Before" data limits studies to post-spill reference-impact designs. Because of the immense geographic scale of DWHOS oiling sampling the entire nearshore ecosystem was not practical; instead, we focus on scaling up geographically focused studies and extending the findings of lab toxicity studies to field conditions

We chose seven taxa that are integral to nearshore ecosystems in the northcentral Gulf of Mexico and represent a taxonomic and trophic range: Amphipods, Marsh Periwinkle (*Littorina littoraria*), Brown Shrimp (*Farfantepenaeus aztecus*) and White Shrimp (*Liptopenaeus setiferus*), Southern Flounder (*Paralichthys lethostigma*), Gulf Killifish (*Funchulus grandis*), and Red Drum (*Sciaenops ocellatus*). To estimate injury, we used the results of geographically explicit field studies that employed shoreline oiling categories as oil exposure gradients (Periwinkle, Brown and White Shrimp) or laboratory-based toxicity studies applying field-collected or oiled spiked sediments to assess lethal or sublethal (growth, fecundity) endpoints for the seven taxa.

#### 1. Marsh Periwinkle Snails



Marsh periwinkles, a salt marsh snail, are widely distributed, abundant, and conspicuous grazers on algae and fungi that grow on the stems and leaves of marsh plants and on soils. Through their grazing activities, marsh

periwinkles facilitate the production of organic matter, nutrient cycling, and marsh-estuarine food chains. They are vulnerable to oiling impacts because they are closely associated with the marsh substrate and emergent salt marsh vegetation, especially *Spartina alterniflora*.

#### A. Overview of Field Study

A field study conducted in Fall 2011 in mainland marshes in coastal Louisiana assessed oil impacts of HP oiling on marsh periwinkles. Three types of study sites were selected for comparative sampling: 1) sites with HP oiling (HP) where cleanup actions were also conducted, 2) sites with HP oiling without cleanup actions, and 3) reference sites where no oil was observed during marsh surveys (see Nixon et al. 2015 for details on shoreline oiling observations). Marsh periwinkle density and shell lengths at sites with HP oiling were compared to snails in reference conditions. Periwinkle density and size were also evaluated between HP oiled sites with and without shoreline cleanup treatments. 32 marsh edge sampling stations were located (~2 m) from the shoreline, and 35 interior stations were located an average of ~9 m from the shoreline. An additional zone of interior marsh was located inland of observed oil penetration, where stations were placed an average of 69 feet (21 m) from the shoreline. No effects on periwinkles were observed in the zone located inland of observed oiling (Zengel et al. in review).

#### **B.** Injury Scaling

Zengel et al. (In Review) documented decreases of periwinkles in zone 1 (marsh edge to 6 m) and zone 2 (6 - 15 m zone) in areas of heavy persistent oiling in 2011. Compared to

reference areas, adult sized L. littoraria density was lower by 25 snails m<sup>-2</sup> in zone 1 and 54 snails m<sup>-2</sup> in zone 2. Standard errors on the loss terms were +/- 14 snails in zone 1 and 29 snails in zone 2. Differences in recruits (0-8 mm snails) were not detected by the study. After discussion with the authors of the study, we attribute this to selectivity of the collection methodology because collection was based on visual census of the exposed area. Small snails are difficult to collect as they hide in blades of saltmarsh plants and detritus. Subadult snails (8-14 mm) were largely missing from the study site, we interpret this absence as a result of a combination of the selectivity of the sampling methodology and failed recruitment in 2010 (samples were not collected until 2011, 1 year after the spill). To determine the density of snails that should have been in these missing two size classes (0 yr and 1 yr old snails), we assumed a stable age distribution and predicted the abundance of these two size classes in the impacted areas. To derive this age distribution we assumed that the delta density measured by Zengel et al. (In Review) reflected the density of 2-10 year old snails (see Figure 1). The 10 year maximum age was based on professional judgement of experts (C. Montague and the authors). From this T<sub>max</sub>, a natural mortality rate of 0.422 was estimated from Hoenig's (1983) widely used equation to estimate natural mortality based on longevity,  $M = 4.22/T_{max}$ .

#### C. Results

Production foregone was estimated from the partitioning of the injury estimate in Zengel et al. (In Review) into a matrix of numbers at age derived from the life table presented in Table 1. No von Bertalanffy growth has been published for Marsh Periwinkles; consequently, we derived our own von Bertalanffy curve based on professional judgement of size at age estimates. Specifically, we estimated the shell length of 0.5- (5 mm), 1- (9 mm), 2- (15 mm), 3- (20 mm), 4- (23 mm) and 5- (26 mm) year old snails as well as an age 10 snail (30 mm) and solved for K

(growth coefficient) and  $L_{inf}$  (maximum size) of the von Bertalanffy curve,  $L(t) = L_{inf} *[1 - exp(-K*(t-t_0))])$ , holding To (t at zero length) = 0. We estimated best fit of the curve by the residual sums of square estimate, which = 0.732 under our best fit model. We then produced the Von Bertalanffy curve presented in Figure 2 using the estimated  $L_{inf}$ , K, and To. Lost production based on sampling in Zengel et al. (In Review) yielded an estimate of 121.5 g m<sup>-2</sup> (whole wet weight) in zone 1 from the loss of 25 age-2-10 snails plus 14 age-1 snails (estimated according to the stable age distribution). For zone 2, lost production of 54 age 2-10 plus 28 age 1 snails was estimated to be 283.8 g m<sup>-2</sup>. This lost production includes both the weight of snails estimated at the time they were killed as well as the production (weight gain) these snails would have achieved if they lived through their predicted natural life span.

The production reduction calculated based on the density decline observed in Zengel et al. (In Review) was applied over a 6 m wide "edge" zone (Zone 1), and a 9 m wide "inland" zone (Zone 2) and multiplied by the length of HP oiled, vegetated shoreline (62 km). The effect of oiling would equate to a total loss of 204 metric tons (whole wet weight) from the marsh system. These effects would be expected over 39 miles (62 km) of HP oiled shorelines in Louisiana mainland herbaceous marshes. Production lost at the time of the kill was estimated to be 106 metric tons. These individuals would have been expected to achieve additional production of 98 metric tons (Table 3).

Sources of uncertainty in this calculation include variations in interior zone widths, variation in the number of periwinkles found at each station, uncertainty in the length of shoreline miles oiled (shoreline miles were estimated as a subset of LA heavily persistently oiled miles), and uncertainties in baseline densities of snails, and growth assumptions. The largest source of uncertainty in our growth calculations is the estimate of  $T_{max}$ , and the fate of the

missing young age classes in Zengel et al. (In Review). Other information for the life table came directly from sampling the area to fill the data gap for the length-weight relationships (Powers, unpublished data (see Figure 3). The estimate of  $T_{max}$  at 10 years is based on best professional judgment. Snails are known to live multiple years; however, mark–recapture studies that document the presumed 10-year life span are not available. Because older snails grow little after age 5, our estimate of lost production would change by less than 15% if we were to truncate the age to 5 years. Hence, we believe our estimate is robust to changes in  $T_{max}$  from 5 to 10 years (see Table 4).

Table 1. Key parameters used in the Marsh Periwinkle injury calculation.

Key Parameters	Value		Units	Source
HP Shoreline Distance, (E-W River)		62	Km	Nixon et al. 2015
Width of Zone 1		6	M	Nixon et al. 2015
Width of Zone 2		9	M	Zengel et al. In Review
Reduction in Adult Density Zone 1		-25.57	# m <sup>-2</sup>	Zengel et al. In Review
Reduction in Adult Density Zone 2		-53.77	# m <sup>-2</sup>	Zengel et al. In Review
M		0.422		$M = 4.22/T_{max}$ as in Hoenig
L <sub>inf</sub>		31	mm	
K		0.338	coefficient	Derived from von Bert growth curve
to		0	mm	Set to zero
$T_{max}$		10	yrs	Clay Montague, pers. com.
Whole weight: a (in W = a *L <sup>b</sup> )	(	0.0760	coefficient	Powers et al. unpublished data
Whole weight: b (in W = $a *L^b$ )	(	0.1583	coefficient	Powers et al. unpublished data

Table 2. Estimated number of Marsh Periwinkle snails lost based on Zengel et al. 2015 study. The upper table gives estimates directly from sampling of large snails. The lower table gives estimates from direct sampling along with the missing smaller age classes resulting from the selectivity of the sampling gear and failed recruitment in 2010.

		A. Injury es	timate direc	tly from sampl	ing in Zengel et al. (I	n Review)			
				Heavier					
				Persitent					
		Change in	Change in	shoreline	Area of zone (6 m	Nummber of	Number of	Number of	Total loss
Zone	Change in adult	subadult	recruit	distance (km)	& 9 m width)	adult snails lost	subadult	recruits	(number of snails)
Measured zone 1	(26)	(2)	(2)	62	372,000	(9,512,040)	(744,000)	-	(10,256,040)
Measured zone 2	(54)	-	-	62	558,000	(30,003,660)	-	-	(30,003,660)
Total						(39,515,700)	(744,000)	-	(40,259,700)
			B. Injury es	timate includin	g subadult and recru	uits			
				Heavier					
				Persitent					
		Change in	Change in	shoreline	Area of Zone (6	Nummber of	Number of	Number of	Total loss
Zone	Change in adult	subadult	recruit	distance (km)	m & 9 m width)	adult snails lost	subadult	recruits	(number of snails)
Estimated zone 1	(26)	(14)	(17)	62	372,000	(9,512,040)	(5,108,410)	(6,308,439)	(20,928,889)
Estimated zone 2	(54)	(29)	(36)	62	558,000	(30,003,660)	(16,113,367)	(19,898,596)	(66,015,624)
Total						(39,515,700)	(21,221,777)	(26,207,035)	(86,944,512)

Table 3. Production foregone of Marsh Periwinkle snails from results of Zengel et al. (In Review).

	Change in snail biomass (whole	Heavier Persitent shoreline distance	Area of zone			Total loss	Direct (metric	Foregone (metric
Scenario	wet weight, g m <sup>-2</sup> )	(km)	width)	Total loss (g)	Total loss (kg)	(metric tons)	tons)	tons)
Estimated zone 1	121.5	62	372,000	45,214,436	45,214	45.21	18.97	26.24
Estimated zone 2	283.8	62	558,000	158,369,979	158,370	158.37	86.80	71.57
Total					203,584	203.58	105.77	97.82

Table 4. Injury to Marsh Periwinkle snails partitioned by age class.

			Production
			foregone
Age	Direct Sampling (#)	+Rec+Subadults (#)	(metric tons)
1	14,066,807	30,378,560	71.13
2	9,224,084	19,920,256	46.64
3	6,048,546	13,062,390	30.59
4	3,966,238	8,565,454	20.06
5	2,600,797	5,616,659	13.15
6	1,705,431	3,683,035	8.62
7	1,118,309	2,415,091	5.66
8	733,313	1,583,658	3.71
9	480,859	1,038,458	2.43
10	315,315	680,952	1.59
Total			203.58

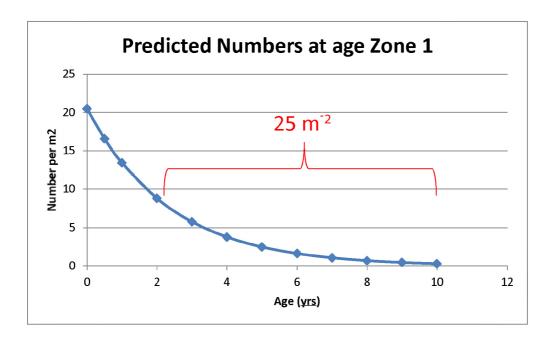


Figure 1. Predicted numbers at age in Zone 1 based on a loss rate of 25 Marsh Periwinkles m<sup>-2</sup> as measured by Zengel et al. (2015) and a natural mortality rate (M) of 0.422.

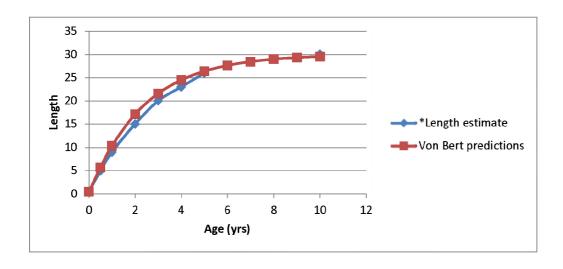


Figure 2. Von Bertalanffy curve for Marsh Periwinkles used in our analysis. See Table 1 for parameter estimates for K, Linf, and To.

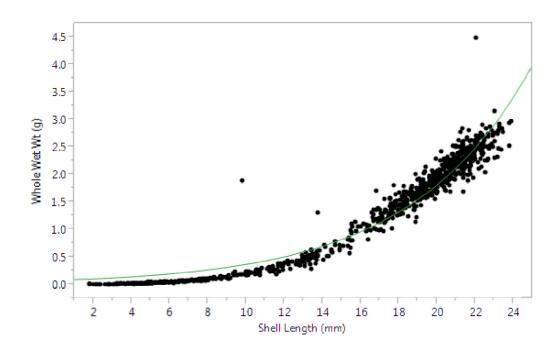


Figure 3. Length (mm) versus whole wet weight of Marsh Periwinkles snails. Data were collected from Barataria Bay, LA (Zengel, unpublished data) and Dauphin Island, AL (Powers, unpublished data). See Table 1 for coefficients for  $W = a*L^b$ ,  $R^2 = 0.942$ .

#### 2. Brown Shrimp and White Shrimp

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White Shrimp (*Liptopenaeus setiferus*) and Brown Shrimp (*Farfantepenaeus aztecus*) are important components of the Gulf of Mexico ecosystem and food web as well as supporting a robust and

valuable commercial fishery. Adult shrimp spawn in open waters of the Gulf of Mexico and as the young develop, they move into estuaries and settle to bottom sediments adjacent to marsh shorelines, where they grow rapidly. The shallow wetland habitats (particularly salt marsh and mangroves) of Barataria Bay and other northern Gulf of Mexico estuaries support high densities of juvenile brown shrimp and white shrimp. Juvenile brown shrimp use the estuary in the winter/spring months, while juvenile white shrimp use the estuary in late summer/fall. Both species are also found at distances of at least 3 m onto the surface of flooded marshes, where they are opportunistic feeders on infauna (polychaete worms in the sediment), plants, and detritus.

#### A. Overview of Field Study

Rozas et al. (2014) conducted experiments in May 2011 on brown shrimp and October 2011 on white shrimp. Shrimp were incubated in cages adjacent to the marsh edge at 25 sites for 1-2 weeks. All experiments were performed in Barataria Bay. Five shoreline oiling categories used in the Rozas et al. (2014) study design were reclassified into four NRDA shoreline oiling categories for purposes of injury assessment (Nixon et al. 2015). Rozas et al. (2014)'s study demonstrates that along HP oiled and H oiled shorelines in "no-food added" treatments, growth of juvenile white shrimp was reduced by 31-46%, and juvenile brown shrimp growth by 27-56% compared to sites that did not experience oiling. Sediment PAH concentrations measured in

Rozas et al. (2014) study were less than 1 ppm, but correlations between shrimp growth reductions and heavy shoreline oiling (where concentrations of PAHs in marsh soils are extremely elevated, 10 -100 ppm) indicate that the observed effects were likely the result of integrated exposure from multiple pathways, including contaminated sediment and chemicals in runoff from oiled marsh habitat in 2011.

#### **B.** Injury Scaling

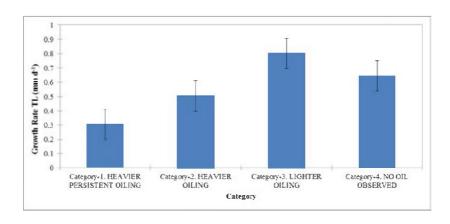
Based on the field results of Rozas et al. (2014), a growth penalty was assigned to each of the HP and H oiling shoreline oiling categories for brown and white shrimp. We used the von Bertalanffy predictions to derive a daily growth estimate under the reference condition; this estimate was similar to that predicted in the "No Oil Observed" areas. The baseline growth rate was then reduced to account for the daily reduction in growth measured in Rozas et al. (2014) experiments for the HP and H oiling conditions (Figure 4). The growth penalty was assessed from months 2-7 of a 12 month life cycle for white and brown shrimp. This period reflected the seasonal time period shrimp are found in tight association with the marsh edge and the flooded marsh surface. In 2010, brown shrimp would have already recruited to the shoreline prior to oil coming on shore, hence, the penalty for brown shrimp in 2010 was applied starting at month 4. For white shrimp, late/summer fall recruit cohorts in 2010 and 2011 would have been exposed to those conditions detailed in Rozas et al. (2014), whose experiments were conducted in the fall of 2011. Brown shrimp in 2011 would have experienced reduced growth for the entire period of marsh fauna use. Application of the growth penalty resulted in distinct length-at-age curves for each of the scenarios modeled: Brown Shrimp 2010, Brown Shrimp 2011, and White shrimp 2010 and 2011 at No Oil Observed (NOO), HP and H shoreline zones (Figures 5 and 6).

Production expected from each of the three zones (HP, H, and NOO) was calculated by predicting numbers at age (month) from initial density of 3-month old shrimp on the marsh edge. This density estimate was derived from a thorough literature review. Post larvae were modeled to settle out of the plankton and into the marsh edge by the end of month 2. At that point, post-larvae (PL) began to experience different growth rates associated with the type of shoreline oiling category they occupied. For Brown Shrimp in 2010, the oiling penalty was introduced in month 4 and lasted through month 7. By month 8 shrimp are foraging away from the marsh edge. The density of PL was used as the initial Ni (number of shrimp at stage (i)) at 2 months; mortality of subsequent stages (months) was predicted by a size dependent mortality equation reported in Adamack et al. (2014). Mortality would be expected to be higher in low-growth scenarios (HP and H zones) compared to baseline (NOO) (Figures 7 and 8). Numbers at age per m² was multiplied by weight gain in each monthly interval, and summed to calculate production (g m²) under each oiling scenario for brown and white shrimp.

Table 5. Parameters utilized in the shrimp modeling studies.

Parameter	Brown Shrimp	White Shrimp	Source
Density of 3-month old	3.63 m <sup>-2</sup>	2.15 m <sup>-2</sup>	Derived from
shrimp in -1 to 50 m marsh			Literature Review.
edge swath			
Density of Post-Larvae in -	39.96 m <sup>-2</sup>	23.7 m <sup>-2</sup>	Derived from life
1 to 50 m marsh edge			history table and
swath			density of 3 month old
			shrimp.
K	1.13	1.13*	Adamack et al. 2011
Linf	191 mm	191 mm*	Adamack et al. 2011
То	-0.294	-0.294*	Adamack et al. 2011
M (size dependent)	$Mi = 53.09*Li^{-1.12}$	$Mi = 53.09*Li^{-1.12}$	Adamack et al. 2011
HP % change in growth	-56% of base	-46% of base	Reanalysis of Rozas
			et al. (2014) data.
H % change in growth	-27% of base	-31% of base	Reanalysis of Rozas
			et al. (2014) data.
Weight to Length	$W = 10.0^{(-5.129 + \log(L) \cdot 3.013)}$	$W = 10.0^{(-5.129 + \log(L) . 3.013)} *$	Adamack et al. 2011
conversions			

\* Values for brown shrimp were used for white shrimp because specific values for white shrimp were not available from the literature.



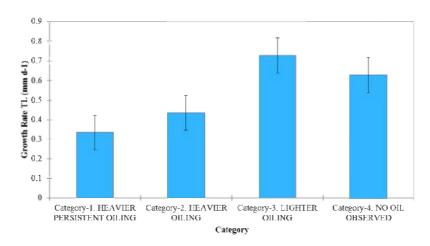


Figure 4. Growth of brown (upper) and white shrimp (lower) in different shoreline oiling categories as measured by Rozas et al. 2014 based on the no-food-added treatment and recalculated for the updated shoreline oiling classification. We examined the production lost from baseline conditions (No oil observed) and compared to HP and heavier oiling shoreline conditions.

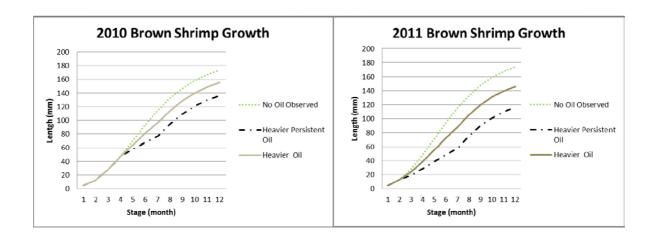


Figure 5. Modeled growth of Brown Shrimp in 2010 and 2011 at "No Oil Observed", Heavier Persistent Oiled, and Heavier Oiled shoreline areas. The calculation was based on the realized growth penalties from data in Rozas et al. (2014).

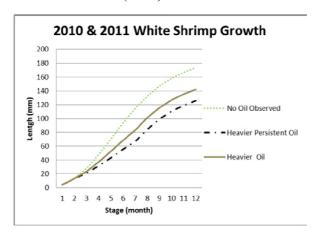


Figure 6. Modeled growth of White Shrimp in 2010 and 2011 at "No Oil Observed", Heavier Persistent Oiled, and Heavier Oiled shorelines. The calculation was based on the realized growth penalties from data in Rozas et al. (2014).

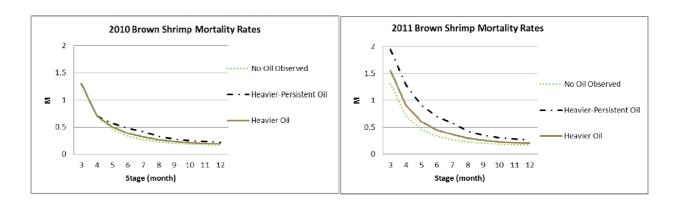


Figure 7. Modeled mortality of Brown Shrimp in 2010 and 2011 at "No Oil Observed", Heavier Persistent Oiled, and Heavier Oiled shorelines. The calculation was areas based on predicted size at age in three different shoreline oiling zones.

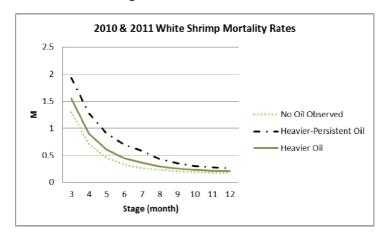


Figure 8. Modeled mortality of White Shrimp in 2010 and 2011 at "No Oil Observed", Heavier Persistent Oiled and Heavier Oiled shorelines. The calculation was based on predicted size at age in three different shoreline oiling zones.

#### C. Results

Reductions in juvenile shrimp growth observed along oiled shorelines would translate directly into fewer adult shrimp, since predation and mortality from other sources is size dependent. The faster the shrimp grow, the better their chances of avoiding predation and surviving to reach adult life stages. Reduced shrimp growth was converted to production per m<sup>2</sup>

and is given in Figure 9a. The difference in life time production estimates of shrimp residing in each zone as juveniles from base line (NOO) and oiled areas (HP and H) was calculated and multiplied by the injury area. The injury area was calculated to include a 1-m wide swath of marsh surface and a 50 m wide area of adjacent submerged sediment, which is the area where shrimp have been observed in prior studies, and the linear shoreline distance of HP and H zones across the spill affected area (see Tables 6 and 7). The effect of oiling would equate to a total loss of 1,176 metric tons (mt) wet weight of brown shrimp from the areas of HP and H oiled marsh system over 2010 and 2011 (Figure 9b). 913 mt of white shrimp production was lost during the same period due to oiling. Oiling effects persisted for at least two years (into fall 2011) along 179 miles of HP and H oiled shoreline in Louisiana and Mississippi. Since PAH concentrations remained elevated in marsh soils into 2012 and 2013, it is likely that reductions in shrimp production persisted longer than simply to 2011 in areas that experienced HP oiling. This injury would continue for as long as HP oiling conditions are present.

Sources of uncertainty in this calculation include variations in interior zone widths, variation in the growth of shrimp in each treatment group, uncertainty in the length of shoreline miles oiled, and variability in baseline densities of shrimp, and growth assumptions.

In addition to the effects of marsh oiling on the growth of juvenile shrimp, summer river water releases and resulting reduced salinity as part of spill response likely reduced juvenile brown shrimp production by affecting benthic prey abundance or through the stress of adapting to lower salinity conditions (Adamack et al. 2012). Adamack et al. (2012) modeled the effects of a late April/May water release production would be 40 to 60 percent less than under baseline conditions where no release would occur during this period. Benthic prey quantity dropped from 60 mg/core to 5 mg/core in areas where salinity was less than 5 ppt (Rozas and Minello 2011)

data used by Adamack et al. (2012). White shrimp juveniles would not have been affected by these freshwater conditions since most movement of juvenile white shrimp into marshes occurs later in the summer and fall. Reductions in brown shrimp production from river water releases would be expected to occur over an additional area where salinities dropped below 5 parts per thousand in 2010 when compared to prior years (Figure 10). This area was determined by interpolating thousands of salinity values throughout the estuary and comparing 2010 salinities to those in the years prior to the spill (2006-2009) (McDonald et al. 2015, Rouhani and Oehrig 2015b). Estimates of prior year conditions are intended to represent salinity conditions that were likely to have occurred in 2010 had the release of river water not occurred as part of the response action.

Freshwater conditions may have also affected other animals in addition to shrimp. Rose et al. (2014) suggested negative impacts of river water releases on other estuarine dependent fishes and invertebrates, but did not quantify the effects in terms of lost production. Rose et al. (2014) included in their study a specific model of the river water releases during the Deepwater Horizon (i.e. "Oil Spill" scenario). The results of Rose et al. (2014) illustrate that the effect demonstrated for brown shrimp likely extend to other fauna.

# Production of Shrimp g m<sup>-2</sup> 1.HEAVIER PERSISTENT OILING 2.HEAVIER OILING 4.NO OIL OBSERVED White Shrimp 2010 White Shrimp 2011 Brown Shrimp 2011 Brown Shrimp 2011

Figure 9a. Shrimp production (g  $m^{-2}$ ) for shrimp residing in Heavier Persistent Oiling, Heavier Oiling, and No Oil Observed zones. Production is lifetime expected production stemming from Shrimp Post Larvae recruits in each zone.

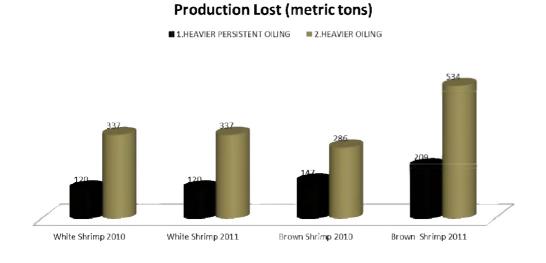


Figure 9b. Estimated production lost due to oiling for White and Brown Shrimp in HP Oiled and Heavier Oiled areas.

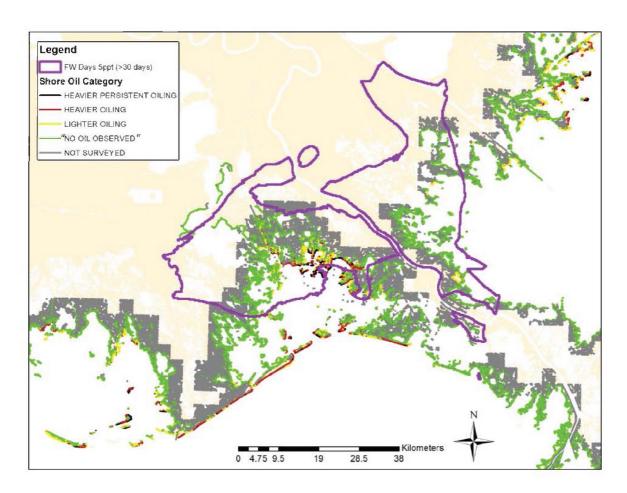


Figure 10. Extent of the impacts of the summer river water releases in response to the approaching *Deepwater Horizon* oil. Salinity levels in the areas outlined in purple were much lower for much longer than in a typical year (Rouhani and Oehrig 2015b).

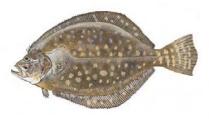
Table 6. Injury calculations for White Shrimp due to oiling of marsh edge habitats.

2010	Shoreline Length (km)	Nearshore Area (km2)	Adjusted Nearshore Area (km2)	Production per m2	Production Lost g/m2	Production Lost kg/km2	Production Lost kg	Production Lost (mt)
Habitat/Oiling								
1.Marsh/Mangrove								
1.HEAVIER PERSISTENT OILING	67.0	3.4	3.4	2.25	-35.67	-35673.40	-119505.90	-119.51
2.HEAVIER OILING	221.0	11.1	11.1	7.38	-30.53	-30534.55	-337406.73	-337.41
4.NO OIL OBSERVED	3069.8	153.5	153.5	37.92	0.00	0.00	0.00	0.00
Total								-456.91
2011	Shoreline Length	Nearshore	Adjusted Nearshore	Production	Production	Production	Production	Production Lost
	(km)	Area (km2)	Area (km2)	per m2	Lost g/m2	Lost kg/km2	Lost kg	(mt)
Habitat/Oiling	(KM)	Area (km2)	Area (km2)	per m2	Lost g/m2	Lost kg/km2	Lost kg	(mt)
	(KM)	Area (km2)	Area (km2)	per m2	Lost g/m2	Lost kg/km2	Lost kg	(mt)
	(km) 67.0	Area (km2)	Area (km2) 3.4	per m2 2.25	Lost g/m2 -35.67	-35673.40	Lost kg -119505.90	(mt) -119.51
1.Marsh/Mangrove					-35.67			, ,
1.Marsh/Mangrove 1.HEAVIER PERSISTENT OILING	67.0	3.4	3.4	2.25	-35.67 -30.53	-35673.40	-119505.90	-119.51
2.HEAVIER OILING	67.0 221.0	3.4 11.1	3.4 11.1	<b>2.25</b> 7.38	-35.67 -30.53	-35673.40 -30534.55	-119505.90 -337406.73	-119.51 -337.41

Table 7. Injury calculations for Brown Shrimp due to oiling of marsh edge habitats.

2010	Shoreline Length (km)	Nearshore Area (km2)	Adjusted Nearshore Area (km2)	Production per m2	Production Lost g/m2	Production Lost kg/km2	Total Production Lost kg	Total Production Lost (mt)
Habitat/Oiling								
1.Marsh/Mangrove								
1.HEAVIER PERSISTENT OILIN	67.0	3.4	3.4	20.05	-43.94	-43941.07	-147202.58	-147.20
2.HEAVIER OILING	221.0	11.1	11.1	38.13	-25.87	-25867.18	-285832.28	-285.83
4.NO OIL OBSERVED	3069.8	153.5	153.5	64.00	0.00	0.00	0.00	0.00
Total								-433.03
2011	Shoreline Length (km)	Nearshore Area (km2)	Adjusted Nearshore Area (km2)	Production per m2	Production Lost g/m2	Production Lost kg/km2	Total Production Lost kg	Total Production Lost (mt)
Habitat/Oiling					<u> </u>	<u> </u>		
1.Marsh/Mangrove								
1.HEAVIER PERSISTENT OILIN	67.0	3.4	3.4	1.57	-62.42	-62421.51	-209112.08	-209.11
	224.0	11.1	11.1	15.65	-48.35	-48345.12	-534213.54	-534.21
2.HEAVIER OILING	221.0	11.1						
2.HEAVIER OILING 4.NO OIL OBSERVED	3069.8	153.5		64.00	0.00	0.00	0.00	0.00
				64.00	0.00	0.00	0.00	0.00 -743.33

#### 3. Southern Flounder



Flounder are a key predator in marsh ecosystems. Southern Flounder (*Paralichthys lethostigma*) use the surfaces of flooded shallow salt marsh, brackish marsh, mangrove, *Phragmites*, and other coastal habitats throughout the northern Gulf of Mexico,

and their close association with sediment makes them vulnerable to PAHs in marsh soils and submerged sediments. Southern Flounder spend most of their lives associated with bottom sediments. Male flounder have a lower max age and reach smaller sizes than females. During their first year of life, young flounder of both sexes settle in bays and estuaries in the late winter to early spring, where they move onto flooded marsh surfaces to feed. Juvenile flounder eat small fish (including *Fundulus*), crustaceans (including amphipods and juvenile shrimp), and polychaetes. Adult Southern Flounder leave the bays during the fall to spawn in open waters of the Gulf of Mexico.

The high productivity of predators in nearshore marsh environments has been closely linked to the frequent tidal and wind-driven inundation of marsh habitats. These extended hydroperiods of inundation allow fish and mobile invertebrates to forage in the refuge of a structured environment with high prey biomass (Minello et al. 2012). This behavior of fish and mobile invertebrates also exposes them to oil that has been deposited in marsh soils. Inundation of marshes exposed to oiling is frequent (> 64% of the time) (Oehrig et al. 2015). As part of the coastal wetland vegetation study (Rouhani et al. 2015), PAH concentrations were measured in marsh surface soil samples taken at three distances from the vegetation edge between fall 2010 and fall 2013.

#### A. Overview of Laboratory Study

Laboratory studies were conducted to evaluate the effect of Macondo oil on juvenile flounder growth over 32 days of exposure (Brown-Peterson et al. 2015). Juvenile Southern Flounder (1.8-3.5 cm) were placed on sediments spiked with weathered MC252 crude oil over a range of concentrations representing those found at oiled marsh sites. Juvenile Southern Flounder exposed to oiled sediments put on less weight and reached smaller sizes than fish exposed to clean sediment (Figure 11).

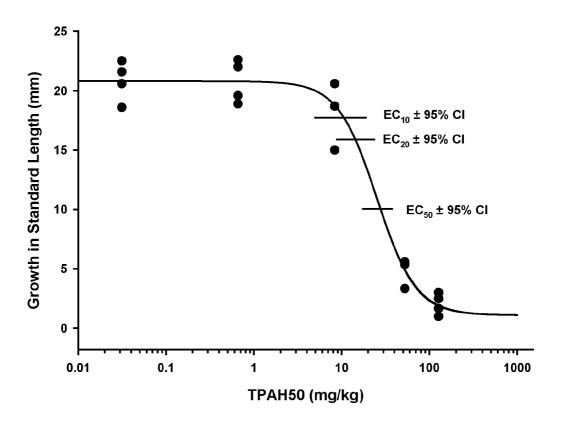


Figure 11. Growth in juvenile southern flounder after a 32-day exposure to sediments spiked with Slick B oil. The EC10, EC20 and EC50 values (95% confidence interval) are 8.4 (5.0, 18.6), 12.8 (8.8, 23.7), 26.7 (17.6, 37.4) mg/kg TPAH50, respectively (Brown-Peterson et al. 2015, see Test 113 Morris et al. 2015).

#### **B.** Injury Scaling

Reduced Southern Flounder growth was converted to lost production using literature values of juvenile Southern Flounder densities on the marsh surface (0.06 m<sup>-2</sup>), the total area of marsh used by juvenile flounder with oil conditions similar to those under which growth was observed to be reduced, and growth/survival relationships. Specifically, von Bertalanffy growth curves were derived for baseline condition and for HP and H conditions that would be expected based on PAH concentrations. For oiling conditions, a threshold value for assessing a "growth penalty" for the first 6 months of life, when flounder are intimately associated with the marsh edge and flooded marsh, was determined by the EC20 value reported in Brown-Peterson et al. (2015). If the PAH concentrations in any of the shoreline oiling categorical zones (Heavy Persistent, Heavier, Lighter) exceeded the EC20 value, then the weighted average PAH concentration of that zone (Rouhani et al. 2015) was used to predict the decreased growth from the baseline conditions (Table 8). PAH concentrations were available for each year (2010-2013) and for each shoreline oiling category. The decreased growth rate was then used to predict the size of a 0.5 year old flounder exposed to that specific PAH concentration for the first six months of life. The growth coefficient (K) in the von Bertalanffy growth curve was then adjusted to pass through the point of reduced growth and then approach the prescribed Linf as in the baseline condition (Table 9, Figure 12).

After a size-at-age matrix was created for baseline and HP and H oiling conditions, we predicted number of individuals surviving through each age up to sex specific  $T_{max}$  through a standard life table analysis. Published estimates of instantaneous mortality (M) rate (M = 1.08 for male; M = 0.55 for female) were used and scaled (according to Lorenzen 2000; Brodziak et al. 2011) to account for size-dependent mortality using the predicted size at age from the von

Bertalanffy equations. The Lorenzen function used took the form where Mi = M \* Li/Lr; where Mi is the age or size specific mortality and Li is the length at i and Lr is the length of the reference value (defined as a fully selected individual for the gear type used to determine M) (Figure 13). Lr was estimated based on professional judgment but was never greater than size at maturity. To complete the numbers-at-age matrix, an initial density was determined from a synthesis of the available literature. The initial density (0.06 m<sup>-2</sup> with a 1:1 male:female ratio) was chosen to represent 0.5 yr olds based on size targeted by the sampling gear used in the literature reviews (see methods in Peterson et al. 2003). Weight at each age class (Wi) was determined by the weight to length conversion, where  $Wi = a*Li^b$ , and a and b are species and sex specific coefficients and Li is the length at i. For females a = 0.0002 and b = 3.314; and for males a = 0.000906 and b = 2.5723 with length in cm and weight in g. Production in wet weight was determined by Pi = (Wi-W(i-1))\*Ni. Production lost was then the sum over all age classes for the oil category in question minus the baseline prediction. We chose to sum all ages under the assumption that changes in production for the life time of the fish would be attributable to greater mortality as a result of slower growth and slower growth and hence less weight gain.

Table 8. Growth penalties for Southern Flounder expected based on laboratory toxicity results (see Figure 11) and exposure to inundated marsh sediments during their first six months of life. HP = Heavier Persistent Oiled and H = Heavier Oiled. PAH concentrations are based on values presented in Rouhani et al. 2015. For 2011 Spring PAH concentrations are reported for 2012 and 2013 only fall concentrations were available.

Year	HP – PAH Avg.	HP – Growth Penalty	H- PAH Avg.	H- Growth penalty
2011	130 ppm	-90%	5 ppm	о%
2012	126 ppm	-90%	< 2ppm	0%
2013	32 ppm	-32%	< 2ppm	0%

Table 9. Changes to von Bertalanffy growth parameters based on reduced growth of Southern Flounder exposed to shoreline oiling during the first six months of their life. See Table 8 for derivation of growth penalties. HP = Heavier Persistent Oiled; Normal is the baseline conditions expected at "No Oil Observed" areas.

Parameters	Length	Length	Length	Length
	(Normal)	HP 2011	HP 2012	HP 2013
		90% Growth	90% Growth	32% Growth
		Penalty	Penalty	Penalty
K	0.38	0.06	0.06	0.28
L <sub>inf</sub> (cm)	66	66	66	66
to	0	0	0	0

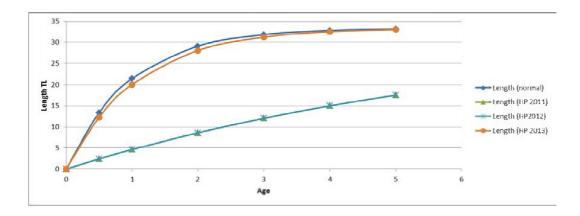


Figure 12. Predicted growth curves based on exposure of Southern Flounder males to different shoreline oiling conditions. HP = Heavier Persistent Oiled; Normal is the baseline conditions expected at "No Oil Observed" areas.

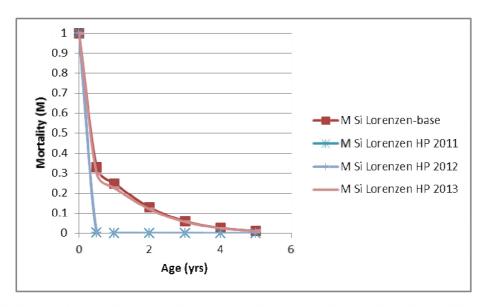


Figure 13. Predicted mortality at age for Southern Flounder males resulting from differential growth experienced under different shoreline oiling and years. Different mortality rates would be expected for animals that grow at different rates, particularly early in their life history.

#### C. Results

Flounder are a key predator in marsh ecosystems. PAH concentrations at H and HP oiled Louisiana mainland herbaceous shorelines would be expected to reduce juvenile flounder growth by 31-90%. A total of 40 metric tons wet weight of flounder was lost where PAH concentrations in marsh soils exceeded 12.6 ppm. Reduced flounder production persisted through 2013 and would be expected to continue in oiled marshes until soil concentrations drop below 8 ppm.

Oil stranded on marsh shorelines beginning in early summer of 2010. By this time, Southern Flounder juveniles would have grown substantially. In the spring of 2011, when the adult fish that survived 2010 oiling conditions spawned, PAH concentrations at HP oiled mainland herbaceous shorelines in Louisiana reduced juvenile flounder growth by 31-90% as compared to conditions where no shoreline oiling was observed (Table 10 and 11). In 2012 and

2013, HP oiled marsh conditions reduced flounder growth - a 90% reduction in growth in 2012 and a 32% reduction in growth in 2013.

Table 10. Estimated production (wet weight g m<sup>-2</sup>) of Southern Flounder juveniles settling into different areas of varying shoreline oiling. HP = Heavier Persistent Oiled; H = Heavier Oiled; L = Lighter Oiled; and Normal is the baseline conditions expected at No Oil Observed areas.

		Production (g/m2)		
Year	HP	Н	L	Normal
2011	0.08	-	-	54.06
2012	0.08	-	-	54.06
2013	33.83	-	-	54.06

Table 11. Change in production relative to normal baseline conditions for Southern Flounder resulting from juveniles settling into vegetated shorelines under different oiling intensities. HP = Heavier Persistent Oiled; H = Heavier Oiled; L = Lighter Oiled; and Normal is the baseline conditions expected at No Oil Observed areas.

		Change in	1	
		Production	n	
Year	HP	Н	L	Normal
2011	-99.85%	-	-	0.00%
2012	-99.85%	-	-	0.00%
2013	-37.43%	-	-	0.00%

A total of 40 mt wet weight of Southern Flounder (Table 12) was lost due to oiling of mainland herbaceous salt marsh in Louisiana where concentrations of PAHs in marsh soils exceed 12.6 ppm, which was used as the threshold concentration for this analysis. The Flounder loss occurred over 39 miles of HP oiled mainland herbaceous shoreline in Louisiana from 2011 through 2013.

Table 12. Summary of injury calculations for Southern Flounder resulting from juveniles exposure to inundated marsh sediments at Heavier Persistent and Heavier Oiled sites following the DWHOS.

Oiling		Change in	Width of	Linear Km	Total Injury	Metric
Category	Year	production	zone (m)	shoreline*	kg	tons
HP	2011	-53.98	5	62	(16,735.12)	(16.74)
НР	2012	-53.98	5	62	(16,735.12)	(16.74)
НР	2013	-20.24	5	62	(6,273.51)	(6.27)
Total					(39,743.75)	(39.74)

Sources of uncertainty in this calculation include variations in concentration of PAHs in HP zone, variations in zone widths, variation in response of the animals in the laboratory toxicity test, uncertainty in the length of shoreline miles, and uncertainties in baseline densities of flounder, growth, and survival.

#### 4. Red Drum



As a nearshore species, the Red Drum (*Sciaenops* ocellatus) is distributed over a wide range of habitats including estuaries, river mouths, bays, sandy bottoms,

mud flats, sea grass beds, oyster reef, and surf zones. Adults generally spawn near estuary inlets during late summer and fall when they reach 4-6 years old. After a brief planktonic period (4-6 weeks), currents carry young drum to estuaries and near-shore areas where they settle into structurally complex habitats like marshes for shelter and forage while they grow (Levin and Stunz 2005). While adjacent to marsh shorelines in the late summer and early fall of 2010, juvenile Red Drum were exposed to oil. As Red Drum mature (2-3 years), they tend to leave the close association with structured habitats and move into open coastal waters. Juvenile Red Drum eat small crustaceans and marine worms, and later, small fish. Juvenile Red Drum are eaten by birds of prey and larger fish.

#### A. Overview of Laboratory Studies

Laboratory studies were conducted to evaluate the effect of Macondo oil on juvenile drum growth over 13 days of exposure (Figure 14). 2.3-3.4 cm juvenile Red Drum were placed on sediments spiked with weathered Macondo oil over a range of concentrations representing those found at oiled marsh sites. Juvenile drum exposed to oil put on less weight and reached smaller sizes than fish exposed to clean sediment (Figure 14).

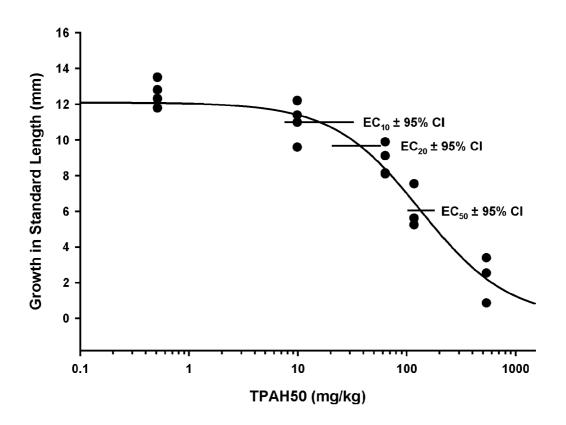


Figure 14. Growth in juvenile red drum after a 13-day exposure to sediments spiked with Slick B oil. The EC10, EC20 and EC50 values (95% confidence interval) are 17.5 (7.62, 32.2), 37.1 (20.9, 56.8), and 134 (103, 178) mg/kg TPAH50, respectively (Morris et al. 2015). (Test 654)

# **B.** Injury Scaling

The overall methodology was similar to that of juvenile Southern Flounder. Length-atage was adjusted as a function of the average PAH concentrations measured in HP areas. No other shoreline categories had average concentrations that exceeded the EC 10 or EC20 thresholds. Von Bertalanffy growth curves were derived for baseline condition and for growth conditions that would be expected in HP Oiled areas based on average PAH concentrations in the Fall of each year. For oiling conditions, a threshold value for assessing a "growth penalty" for the first 6 months of life, when Red Drum are intimately associated with the marsh edge and inundated marsh was established that corresponded to the EC20. Table 13 gives the percentage reduction in growth expected based on the average PAH concentrations measured in marsh soils as well as the dose response pattern in Figure 14. The decreased growth rate was then used to predict the size of a 0.5 year old Red Drum exposed to that specific PAH concentration. The growth coefficient (K) in the Von Bertalanffy growth curve was then adjusted to pass through the point of reduced growth, then approach the prescribed L<sub>inf</sub> as in the baseline condition (Table 16, Figure 15) as fish aged to T<sub>max</sub> (set at 42 years for the calculations). Because differences in size at age are small between male and females, we chose to use a combined male-female growth curve for each scenario modeled.

Table 15. Growth penalties predicted for red drum based on laboratory toxicity experiments and average PAH concentrations measured on marsh soils in Heavier Persistent Marsh Soils. PAH concentrations for fall of each year are given in ppm and details in Rouhani et al. 2015.

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Year	Zone	PAH ppm	Change in growth for first 6 months
2010	НР	129	47%
2011	HP	96	44%
2012	НР	126	47%

After a size-at-age matrix was created for baseline and oiling conditions, we predicted number of individuals in each age class until Tmax through a standard life table analysis. Published estimates of instantaneous mortality (M = 0.2 for red drum) rate were used and scaled (according to Lorenzen 2000) to account for size-dependent mortality using the predicted size at age from the Von Bertalaffy equations. The Lorenzen function used took the form where Mi = M \* Li/Lr; where Mi is the age or size specific mortality and Li is the length at i and Lr is the length (338 mm) of a fully selected individual for the gear type used to determine M (Figure 16). Lr was estimated based on professional judgment but was never greater than size at maturity. To complete the number at age matrix, an initial density was determined from a synthesis of the available literature (see Appendix 1). The initial density was chosen to represent 0.5 yr olds (for red drum this density = 0.3 red drum m<sup>-2</sup>) based on size targeted by the sampling gear used in the literature reviews (see methods in Peterson et al. 2003b). Weight at each age class (Wi) was determined by the weight to length conversion, where Wi = a\*Li<sup>b</sup>, where a and b are speciesspecific coefficients and Li is the length at i (a = 0.000015241 and b = 2.94). Production in wet weight was determined by Pi = (Wi-W(i-1))\*Ni. Production lost was then the sum of all age classes for the oil category in question minus the baseline prediction. We chose to sum all ages under the assumption that changes in production for the lifespan of the fish would be attributable

to either greater mortality as a result of slower growth or slower growth and hence weight gain as a function of degraded juvenile nursery habitat (see rationale in Peterson et al. 2003b).

Table 16. Von Bertalanffy growth parameters used in the determination of size at age relationships for red drum under normal conditions and red drum exposed as juveniles to elevated PAH concentration in Heavier Persistent (HP) Oiled vegetated shorelines.

	Baseline	Length HP 2010	HP 2011	HP2012
	Normal	47% Growth	44% Growth	47% Growth
Parameters	(Predicted)	Penalty	Penalty	Penalty
K	0.22	0.11	0.13	0.11
Linf	950	950	950	950
t0	0	0	0	0

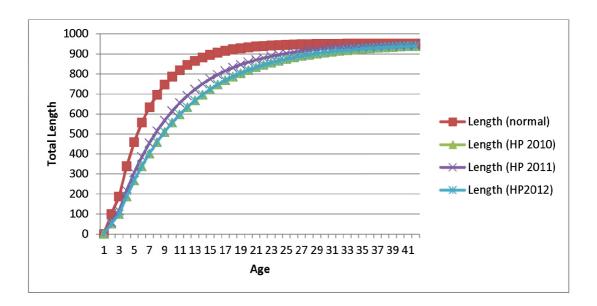


Figure 15. Length at age relationships (Von Bertalanffy Curves) for red drum exposed to different Heavier Persistent (HP) oiling conditions from 2010-2012.

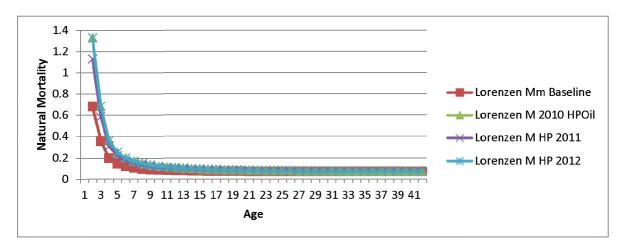


Figure 16. Predicted natural mortality at age relationships for Red Drum exposed to different Heavier Persistent oiling conditions from 2010-2013.

### C. Results

The reduction in growth observed in Red Drum exposed to PAH concentrations similar to conditions at HP oiled marsh sites would translate into fewer adults since small fish suffer higher levels of predation. As was the case for Southern Flounder, concentrations in submerged sediment adjacent to the marsh edge were not high enough to be toxic to drum. The marsh edge ecosystem is inundated for extended periods of time (Oehrig et al. 2015), leading to exposure of Red Drum to high concentration of PAH on the marsh soils. Growth "penalties" were assessed for the period that juvenile drum show a high affinity for structured environments (6 months) and exhibit limited home range. PAH concentrations at HP oiled mainland herbaceous shorelines in Louisiana in 2010 would be expected to reduce juvenile drum growth by 47% when compared to conditions where no shoreline oiling was observed (Table 17 and 18). In 2012, HP oiled marsh conditions were still high enough to reduce drum growth by 47%. By 2013, the soil PAH concentration was reduced below the EC 20 value.

Compared to Southern Flounder, Red Drum are much longer lived and reach a larger size and weight. Hence, lost production of Red Drum would be expected to be much greater than Southern Flounder. A total of 563 metric tons wet weight of red drum was lost due to oiling of mainland herbaceous salt marsh in Louisiana where concentrations of PAHs in marsh soils exceed 37 ppm (Table 19). This effect occurred over 39 miles of oiled mainland herbaceous shoreline in Louisiana between 2010 and 2012. Sources of uncertainty in this analysis include variations of concentrations of PAHs in each zone, variations in zone widths, variation in responses of the animals in the toxicity test, uncertainty in the length of shoreline miles oiled, and uncertainties in baseline densities of drum, and growth, and survival assumptions.

Table 17. Production (g m $^{-2}$  wet weight) of red drum resulting from exposure of juveniles to oiled marshes as well as expected baseline conditions (normal). HP = Heavier Persistent Oiled; H = Heavier Oiled; L = Lighter Oiled; and Normal is the baseline conditions expected at No Oil Observed areas.

		g m <sup>-2</sup>		
Year	HP	Н	L	Normal
2010	755.36	-	-	1413.60
2011	912.72	-	-	1413.60
2012	755.36	-	-	1413.60

Table 18. Changes in production of red drum resulting from exposure of juveniles to oiled marshes as well as expected baseline conditions (normal). HP = Heavier Persistent Oiled; H = Heavier Oiled; L = Lighter Oiled; and Normal is the baseline conditions expected at No Oil Observed areas.

		%change		
Year	HP	Н	L	Normal
2010	-47%	-	-	0
2011	-35%	-	-	0.00%
2012	-47%	-	-	0.00%

Table 19. Summary of Injury calculations for red drum exposed to Heavier Persistent oil shoreline conditions.

		Change in				
Oiling		production	Width of	Linear Km		Metric
Category	Year	(g/m2)	zone (m)	shoreline*	Total Injury kg	tons
HP	2010	-658.25	5	62	(204,056.20)	(204.06)
HP	2011	-500.88	5	62	(155,273.89)	(155.27)
HP	2012	-658.25	5	62	(204,056.20)	(204.06)
Total					(563,386.29)	(563.39)

### 5. Gulf Killifish

Fundulus grandis is an important part of marsh food web, among the most abundant Gulf forage fish, preyed upon by wildlife, birds, and many sport fish, including flounder, speckled trout, and red snapper.

# A. Overview of Laboratory and Field Studies

Marsh soil conditions at HP oiled mainland herbaceous salt marsh shorelines in Louisiana reduced successful hatching of *Fundulus* eggs by 68 to 99% when compared to conditions where no oil was observed (Figure 17). As part of the coastal wetland vegetation study mentioned above, PAH concentrations were measured in marsh surface soil samples taken at three distances from the vegetation edge between fall 2010 and fall 2013 (Rouhani et al. 2015). Laboratory studies were conducted laboratory to evaluate the effect of MC252 oil on *Fundulus* egg hatchability. Fertilized eggs were placed above sediments spiked with weathered MC252 oil over a range of concentrations representing those found at oiled marsh sites. Eggs exposed to oiled sediments were significantly less likely to hatch than those exposed to clean sediments reference (Morris et al. 2015).

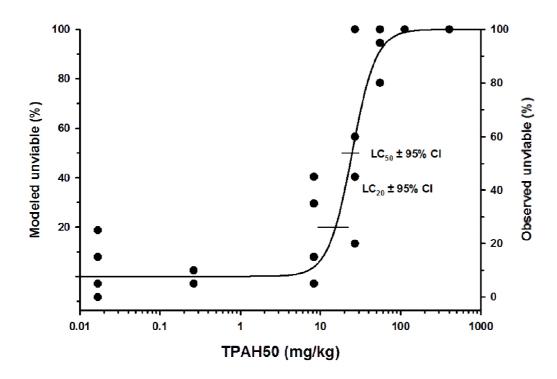


Figure 17. Mortality and non-hatching (unviable) effects on Gulf killifish embryos after a 20-day exposure to suspended sediments spiked with Slick B oil. The LC20 and LC50 values (95% confidence interval) are 15.5 (9.3, 22.1) and 24.8 (18.5, 30.4) mg/kg TPAH50, respectively. These LC50s were calculated using the concentrations of TPAH50 in the sediment from which sediment became suspended during the bioassay and contacted the embryos (Morris et al., 2015). (Test 195)

Table 20. Fecundity (expected mortality of eggs above control) penalties used in the Heavier Persistent (HP) Oiled shoreline areas. PAH concentrations for fall of each year are given in ppm and further details can be found in Rouhani et al. 2015.

			HP
	Н	P-PAH AVG	Mortality
20	10	129	99%
20	)11	96	98%
20	12	130	99%
20	13	32	68%

### **B.** Injury Scaling

Reductions in *Fundulus* egg hatching success associated (Table 20) with exposure to oiled marsh soils would translate directly into fewer adult *Fundulus* available as prey for higher tropic levels. Reduced hatching success was converted to lost production of *Fundulus* adults using literature values of densities of fish eggs on the marsh surface (based on 0.41 eggs per stem and a shoot density of 554 shootsm<sup>-2</sup> in Louisiana herbaceous marsh = 227 eggs per m<sup>-2</sup>), average egg survival to the adult stage (3.2%), the number of spawning events per year (estimated at 2 events), average weight of adults (4.3 g), and a calculation of the total area over which hatching success and production was reduced (shoreline length of each zone x 6 m width). A fecundity "penalty" was applied for areas exceeding concentrations in the laboratory toxicity test associated with reduced hatch success. Injury was calculated for an area to include a 6 m wide swath of mainland herbaceous marsh surface edge habitat where soil PAH concentrations exceed concentrations shown to inhibit hatch success.

### C. Results

Marsh soil conditions at HP oiled Louisiana mainland herbaceous salt marsh reduced successful hatching of *Fundulus* eggs by 68 to 99% when compared to conditions where no oil was observed. Average production (wet weight g m<sup>-2</sup>) in NOO areas was estimated to be 63 g m<sup>-2</sup> with substantial reductions predicted in HP areas from 2010 to 2013 (Figure 18). A total of 84.7 mt wet weight of *Fundulus* was lost due to marsh oiling in mainland herbaceous salt marsh in Louisiana where concentrations of PAHs in marsh soils exceed toxic effects thresholds (Figure 19), which were HP zones in 2010-2013. This effect occurred over 39 miles of HP oiled shoreline from 2010 through 2013. Injury in these areas will persist into the future as long as PAH concentrations in soils remain elevated above concentrations associated with reduced hatch success (approximately 15 ppm total PAHs).

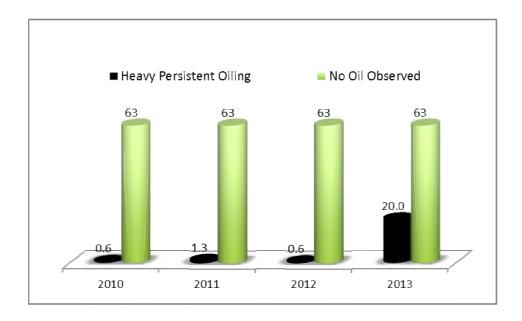


Figure 18. Production (g m<sup>-2</sup> wet weight) of *Fundulus grandis* estimated by shoreline oiling category and year.

Table 21. Summary of injury scaling results and calculations for Fundulus grandis.

	Biomass								
	Change (2010-	Shoreline	Area of Zone					Total lo	oss
Zone	2013) per m <sup>2</sup>	Distance	(6m width)		Total Loss (g)	Total loss	s (kg)	(metric t	ons)
HP	-227.79	62	372,000	-	84,738,976	- 84,73	38.98	- 8	84.74
Total			•	-	84,738,976	- 84,73	38.98	- 8	84.74

Sources of uncertainty in this calculation include variations in concentration of PAHs in each zone, variations in zone widths, variation in response of the animals in the laboratory toxicity test, uncertainty in the length of shoreline miles oiled, and uncertainties in baseline densities of *Fundulus*, and growth, survival, and reproduction assumptions. The injury to *Fundulus* also demonstrates how changes in fecundity associated with the oil spill can affect populations, despite a relatively resistant adult stage.

## 6. Amphipods



Amphipods are representative of organisms living in the soil and sediment that are a primary source of prey for many fish and invertebrates in the food web utilizing the marsh edge. The estuarine benthic amphipod, *Leptocheirus plumulosus*, is a native of east coast

estuaries. Although not a native species to the Gulf of Mexico, it is related both taxonomically and functionally similar to other amphipod crustaceans commonly found in Gulf of Mexico estuaries. *L. plumulosus* is frequently used to evaluate contamination because of its sensitivity and ease of culture and handling. It burrows in bottom sediment and can filter food from water passing over the bottom or graze on algae and detritus on the sediment surface, which makes it susceptible to the effects of marsh oiling. Animals that burrow in sediments, including polychaetes, oligochaetes, and crustaceans, are a primary source of prey for many predators that aggregate near the marsh edge, including white and brown shrimp, *Funchulus*, and flounder (e.g., (McTigue and Zimmerman 1998). *L. plumulosus* was chosen as a representative of sensitive animals that burrow in soils and sediment of mainland herbaceous and back barrier salt marshes, mangroves, and *Phragmites* habitats.

### A. Overview of Laboratory and Field Studies

As part of the coastal wetland vegetation study described above, PAH concentrations were measured in marsh surface soil samples taken at three distances from the vegetation edge between fall 2010 and fall 2013. Amphipods were placed on sediments spiked with weathered Macondo oil for 10 days over a range of TPAH50 concentrations and total organic carbon content (0.4-15%) representing those found at oiled marsh sites. Results from the multiples tests were fitted with a combined curve for calculating LC<sub>20</sub> and LC<sub>50</sub> values (Figure 19; Morris et al. 2015).

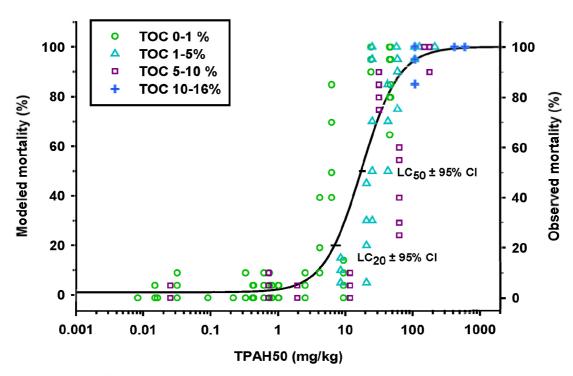


Figure 19. Juvenile amphipod (*Leptocheirus plumulosus*) mortality after a 10-day exposure to sediments spiked with Deepwater Horizon oil in the laboratory (Slick A or B) or contaminated with Deepwater Horizon oil in the field. The LC20 and LC50 values (95% confidence interval) are 7.2 (6.3, 8.2) and 17.9 (16.4, 19.5) mg/kg TPAH50, respectively (Morris et al. 2015). Data are binned according to total organic carbon (TOC) concentrations. (Tests 114, 115, 262, 340, 342, 630/631)

### **B.** Injury Scaling

Marsh soil conditions at heavier and heavier persistently oiled Louisiana mainland herbaceous salt marsh shorelines would be expected to reduce survival of amphipods by 35-95% when compared to shorelines where no oil was observed (Table 22).

Table 22. Mortality penalties for amphipods based on laboratory toxicity results by year and oiling category (see Figure 19). PAH concentrations are given in ppm and further details can be found in Rouhani et al. 2015.

ZONE 1	HP-PAH AVG	HP Mortality	H-PAH AVG	H Mortality
2010	128	95%	12	35%
2011 (Spring)	130	95%	-	-
2011 (Fall)	96	93%	-	-
2012	127	95%	-	-
2013	32	71%	-	-
ZONE 2	HP-PAH AVG	<b>HP Mortality</b>	H-PAH AVG	H Mortality
2010	25	62%	-	-
2011 (Spring)	22	58%		
2011 (Fall)	18	50%	-	-
2012	-	-	-	-
2013	-	_	-	-

Reduced survival from the laboratory toxicity tests was converted to lost amphipod production over time using literature values of amphipod densities on the marsh surface (311 m<sup>-2</sup>; Minello et al. 1994). Assuming eight reproductive events per year, these densities were converted to biomass and then production using a P:B ration of 3.66 (Table 23; Cartes et al. 2002).

Table 23. Equation and key parameters for calculating a production to biomass ratio for amphipods.

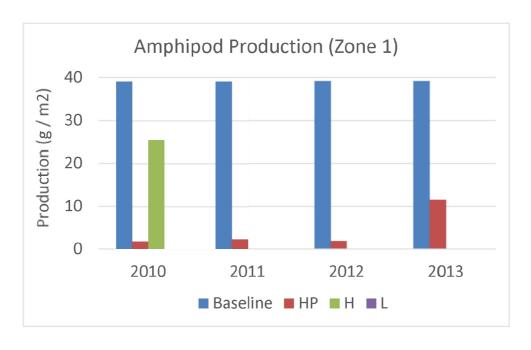
Equation: $\log P/B = 0.349 - 0.203 \log W + 0.020T - 0.362 S_0$	<sub>cap</sub> - 0.119 log Z
T (mean annual temp. C)	28
W (individual dry weight mg)	4.3
S <sub>cap</sub> (swimming capacity)	1
Z (depth / pressure)	0.05
Equation Output	1.30
P/B	3.66

Finally, lost production was calculated for the total area over which survival and production was reduced. For the "heavier persistent" category, injury was calculated for an edge

area to include a 5.8 m wide swath of mainland herbaceous marsh surface and for a 6.1 m wide interior area between 20 and 49 feet (6 and 15 m) from the marsh edge where average measured soil TPAH50 concentrations exceed concentrations shown to inhibit survival. For the "heavier" category, injury was calculated for a 3.7 m wide edge swath (Zone 1) and for a 3.1 m interior area (Zone 2) of mainland herbaceous marsh surface where average measured soil TPAH50 concentrations exceed concentrations shown to inhibit survival. These zone widths encompass the areas over which soil PAH concentrations were measured. The adjacent submerged sediments within 169 feet (50 m) of the marsh edge did not exceed concentrations toxic to amphipods. Concentrations in soil from Delta/*Phragmites* sampling stations did not exceed effects concentrations from the laboratory toxicity test.

### C. Results

Marsh soil conditions at "heavier" and "heavier persistently" oiled mainland herbaceous salt marsh in Louisiana would be expected to reduce survival of amphipods by 35-95% in 2010 when compared to shorelines where no oil was observed. Soils at the edge (zone 1) of heavier persistently oiled marshes would kill 95% of the amphipods using this area based on PAH concentrations measured in 2010. Average production (wet weight g m<sup>-2</sup>) in NOO areas was estimated to be 189 g m<sup>-2</sup> with substantial reductions predicted in HP areas from 2010 to 2013 and H areas in 2010 (Figure 20)



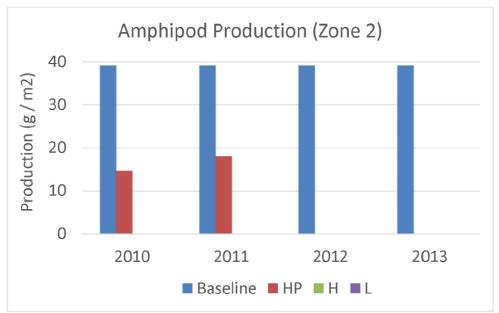


Figure 20. Production (g m<sup>-2</sup> wet weight) of amphipods estimated by shoreline oiling category and year. Zone 1 (edge) is shown in the top panel and Zone 2 (interior) below.

The quantity of amphipods removed from the marsh ecosystem is estimated at 382 MT over 177 miles (285 km) of mainland herbaceous marsh shoreline (Table 24). This effect

occurred in the edge swath (Zone 1) of over 39 miles (62 km) of heavier persistently oiled shoreline from 2010 through 2013 (Figure 20). In the interior (Zone 2), amphipod production was reduced over this shoreline length through 2011. Toxicity of marsh soils to amphipods reduces the availability of this important prey species for fish, crabs, and birds.

Table 24. Summary of injury scaling results and calculations for amphipods.

	HP	Shoreline	Area of Zone			
	Biomass	Distance	(5.8 m & 6.9 m	<b>Total Loss</b>		<b>Total loss</b>
HP	change	(km)	width)	(g)	Total loss (kg)	(MT)
Zone 1	(139.21)	62	359,600	(50,061,562)	(50,062)	(50.06)
Zone 2	(45.61)	62	427,800	(19,512,937)	(19,513)	(19.51)
	Н	Shoreline	Area of Zone			
	Biomass	Distance	(3.7 m & 3.1 m	<b>Total Loss</b>		<b>Total loss</b>
Н	change	(km)	width)	(g)	Total loss (kg)	(MT)
Zone 1	(13.77)	187	691,900	(9,524,795)	(9,525)	(9.52)
Zone 2	0.00	187	579,700	-	-	-
					Total Injury	
					(DW)	(79.10)
		·			<b>-</b>	
					Total Injury	

Sources of uncertainty in this calculation include variations in concentration of PAHs in each zone, variation of interior zone widths, variation in response of the animals in the laboratory toxicity test, uncertainty in the length of shoreline miles oiled, and uncertainties in baseline densities of amphipods, growth ratios, and number of reproductive events per year. Injury in these areas will persist into the future as long as TPAH50 concentrations in heavier persistently oiled marsh soils remain elevated above concentrations associated with reduced survival (approximately 7.2 ppm TPAH50).

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